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LARVAL DEVELOPMENT IN THE ASTEROID *ECHINASTER ECHINOPHORUS*

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Echinaster echinophorus (Lamarck), belonging to the order Spinulosida and family Echinasteridae, is a shallow-water asteroid previously reported from the Caribbean (Bahamas) down to Brazil (Kempf, 1966). This species, having a small central disc and five short radiating arms, occurs in 1 to 2 meters of water on *Diplanthera* and *Thalassia* grass beds, submerged rock deposits and oyster banks. When the breeding season begins, the sea stars move out from beneath grass beds and rocks and clump together in groups of two to six. *Echinaster*, spawning from early April to the end of July, produces large yolky eggs (0.8–1.3 mm diameter) in small numbers (50–250) and exhibit lecithotrophic development with radial, holoblastic, equal cleavage (first three cleavages).

The species *E. echinophorus* appears to either contain several morphologically distinct variants or a complex of closely related species. Morphologically dissimilar animals (spine arrangement, coloration pattern and size) have been observed within the same general habitat to spawn two characteristically different egg types (Atwood, unpublished observations). One egg type (Type 1) ranges from 1.0 to 1.3 mm in diameter, is colored brownish-black and floats throughout early development. The modified brachiolaria becomes a free-swimming larva at about 2 days and settles, attaching to the substratum by means of a larval sucker, by the third day. This developmental type has been reported once before in *Echinaster* found in *Diplanthera* grass beds of Brazil (Kempf, 1966).

The second egg type (Type 2) produced by this species is bright orange, somewhat smaller (0.84–0.88 mm diameter) and settles immediately to the substratum after spawning. These eggs are surrounded by a heavy adhesive material which serves to attach them in groups of 2 to 25 to grass blades, rocks or oyster shells. The modified brachiolaria larvae do not pass through a free-swimming stage.

The purpose of this study is to report on the external morphogenesis and larval growth rate of laboratory cultured *Echinaster* eggs of the second type.

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Materials and Methods

Adult specimens of *Echinaster echinophorus* were collected from shallow-water oyster banks in the Anclote estuary off the coast of Tarpon Springs, Florida, and were maintained in the laboratory in recirculating seawater systems (25° C) until spawning was observed. Eggs were collected from spawning females and placed in aquaria containing dilute sperm suspensions. Males were induced to spawn by injections of radial nerve extracts (Chaet, 1966; Kanatani and Ohguri, 1966; Atwood and Simon, 1971). Larvae were relaxed in 6.5% magnesium chloride, or 0.15% propylene phenoxetol (Goldschmidt Chemical Corp., New York; Owen, 1955; Humason, 1967), fixed in Bouin's solution and stored in 70% ethanol. Natural breeding habits were observed in the field throughout the normal spawning season. All developmental stages were photographed following fixation with the aid of a Nikkormat camera and Wild M-5 dissection microscope.

Sea star size was expressed in terms of R (the distance from the center of the mouth to the tip of the ray), r (the distance from the center of the mouth to the middle of the interbrachial arc), R/r (the ratio between R and r) and wet weight. All arms of each specimen were measured with an ocular micrometer and the average R used. One measurement of r (the largest of the five values) was recorded for each specimen.

Wet weights were measured by removing sea stars from water, holding until most of the fluid had dripped from them, blotting with filter paper and weighing together with any liquid that continued to exude.

Observations

Cleavage occurred rapidly at the laboratory culture temperature of 25° C with the 32-cell stage being reached 6½ hours after fertilization. The young gastrula was formed by the end of the first day. By 48 hours the gastrula had elongated into an oblong larva consisting of a large larval body and a smaller preoral lobe (PL), with rudiments of four larval arms (two dorsal—A, B; and two ventral—C, D) and a central sucker (S) (Fig. 1). Three days after fertilization bulges of the five hydrocoel lobes and the first two pairs of tube feet on each ray were evident on the left side of the larval body of the modified brachiolaria (Fig. 2). The larvae had increased in length from 0.86 to 1.48 mm by the fourth day and begun to flatten laterally. The hydropore (H) appeared on the right side of the larval body by the end of the fourth day (Fig. 3).

Larvae of five days (Fig. 4) had reached a length of 1.53 mm. The bulbous arms increased in size as the centrally located sucker became differentiated. The water ring and radial canals appeared more distinct as bulges for the terminal podia (P) became evident. Both pairs of tube feet on each ray showed signs of growth with the proximal pair being better differentiated. Counting rays in a clockwise direction beginning with the ray at the base of the preoral lobe (Fig. 4), the formation of tube feet paralleled that of the hydrocoelic pouches, with those of rays 3 and 4 developing first, followed by rays 2 and 5, and finally number 1.

By 7 days the tube feet were functional as the juvenile sea star began to move about. Terminal podia (P) were differentiated and exceedingly extendable (Fig. 5). Absorption of the preoral lobe at this stage was evident as it began to fold

toward the oral side of the sea star. Larval arms (A, B, C, D) had reached their maximum size and begun to show stress under the folding process (Fig. 5).

Distinction between the left (B) and right (A) dorsal larval arms became difficult at $7\frac{1}{2}$ days as absorption of the preoral lobe continued and the folding proceeded toward completion. Absorption of the right ventral arm (C) preceded

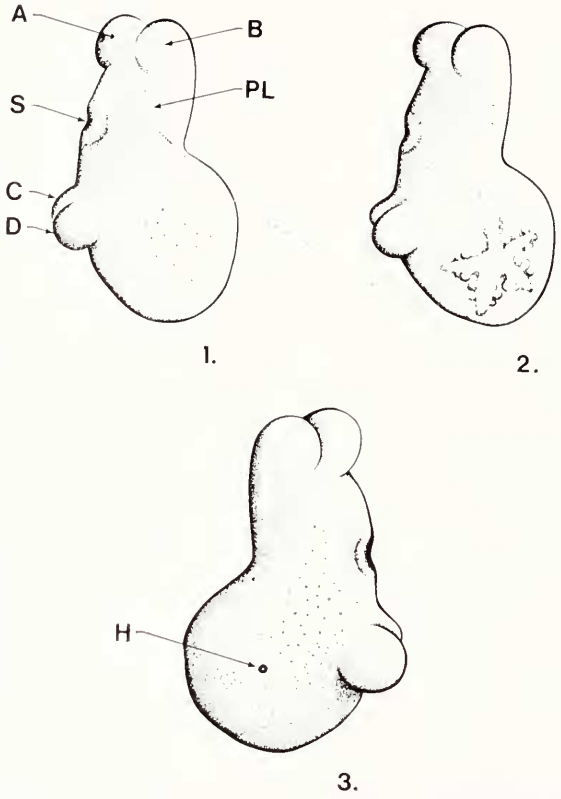


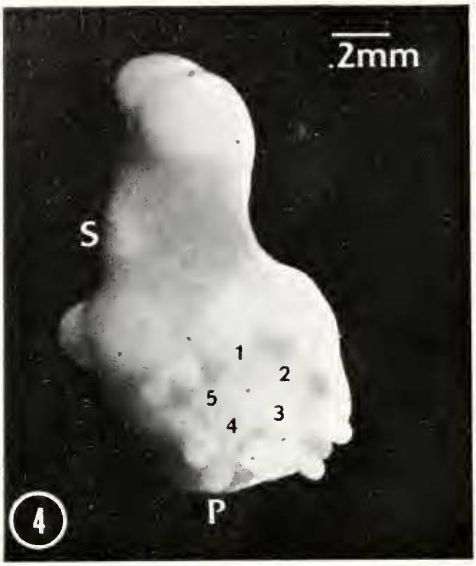
FIGURE 1. Left side (oral) view of a 2-day-old brachiolaria showing the larval right dorsal arm (A), left dorsal arm (B), right ventral arm (C), left ventral arm (D), larval sucker (S) and preoral lobe (PL).

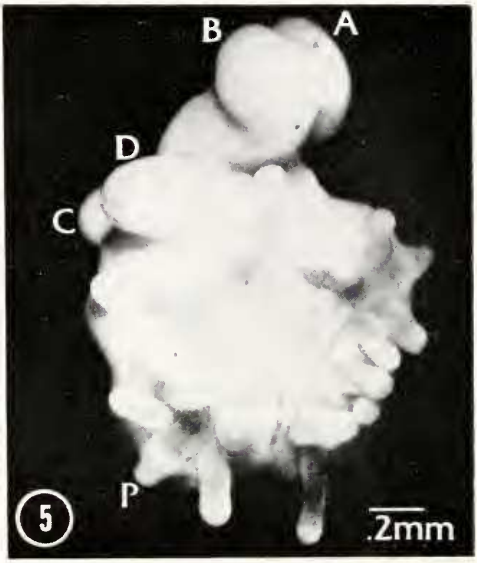
FIGURE 2. Three-day-old brachiolaria showing bulges for the five hydrocoel pouches and first two pairs of tube feet.

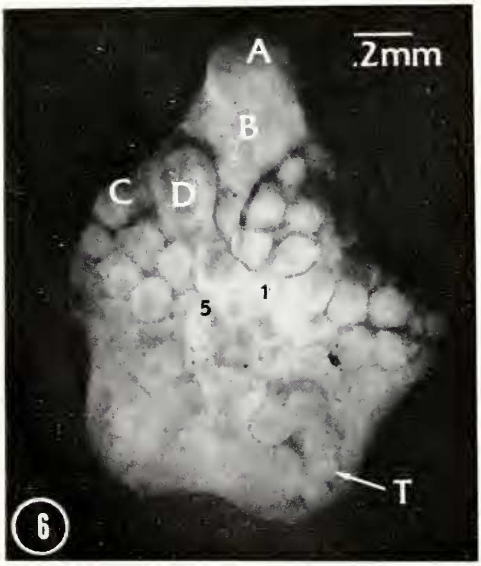
FIGURE 3. Right side (aboral) view of 4-day-old brachiolaria with functional hydropore (H).

that of the left (D). After folding was complete (8 days), the left ventral arm (D) was positioned between the first and fifth rays. The right ventral arm (C) was shifted distally and aboral to the left (Fig. 6). Absorption of the larval sucker appeared to be complete as the third pair of tube feet (T) on each ray appeared on the eighth day (Fig. 6).

The preoral lobe (PL) had been reduced to a small bulge between the first and fifth rays by the tenth day (Fig. 7). A fourth pair of tube feet (T) on each







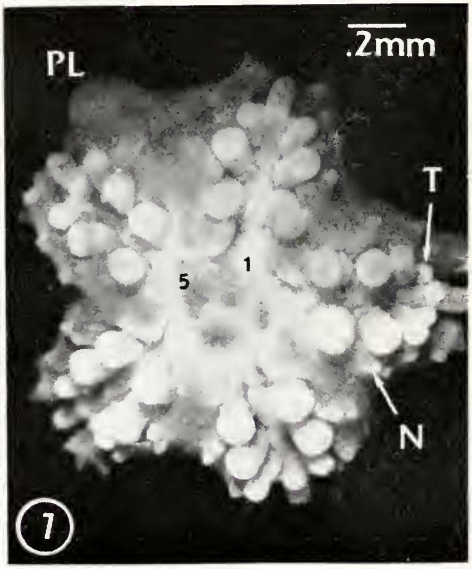


FIGURE 4. Five-day sea star (oral view) with larval sucker (S), bulges for terminal podia (P) and rays 1–5.

FIGURE 5. Preoral lobe folding in 7-day sea star with larval arms (A, B, C, D refer to Fig. 1) and terminal podia (P).

FIGURE 6. Eight-day sea star with larval arms (A, B, C, D refer to Fig. 1), third pair of tube feet (T) and rays 1 and 5.

FIGURE 7. Ten-day-old *Echinaster* juvenile showing remaining preoral lobe (PL), fourth pair of tube feet (T), spines (N) and rays 1 and 5.

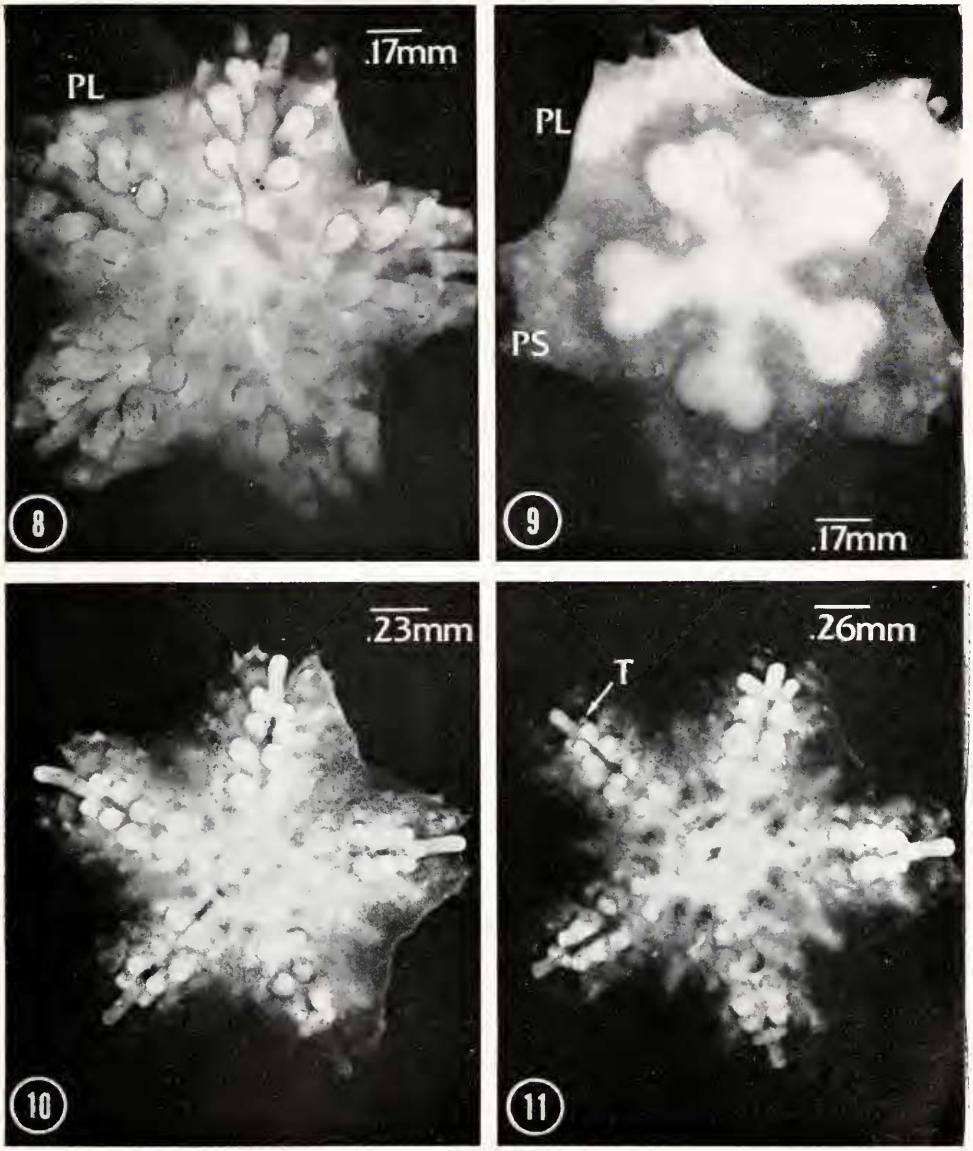


FIGURE 8. *Echinaster* juvenile at 13 days showing small quantity of remaining preoral lobe (PL) tissues.

FIGURE 9. Aboral surface of 13-day sea star with remaining preoral lobe (PL) and light pigment spots (PS).

FIGURE 10. Fourteen-day-old *Echinaster* with newly formed mouth.

FIGURE 11. *Echinaster* at 35 days showing the translucent appearance and the recently developed fifth pair of tube feet (T).

ray formed between the third pair and the terminal podium as spines (N) developed on the oral and aboral surfaces. Spines were larger and more numerous on the oral surface near the terminal portion of each ray (Fig. 7). Thirteen days after fertilization, the preoral lobe (PL) was reduced to a small thickening and light-colored pigment spots (PS) were evident on the oral and aboral surfaces (Figs. 8, 9). The thin membrane sealing the oral region ruptured in 50% of examined juveniles at fourteen days exposing a small functional mouth (Fig. 10). All four pairs of tube feet on each ray were functional at this stage. The anus opened when the larvae were 16 days old. The larval preoral lobe was completely absorbed by this time.

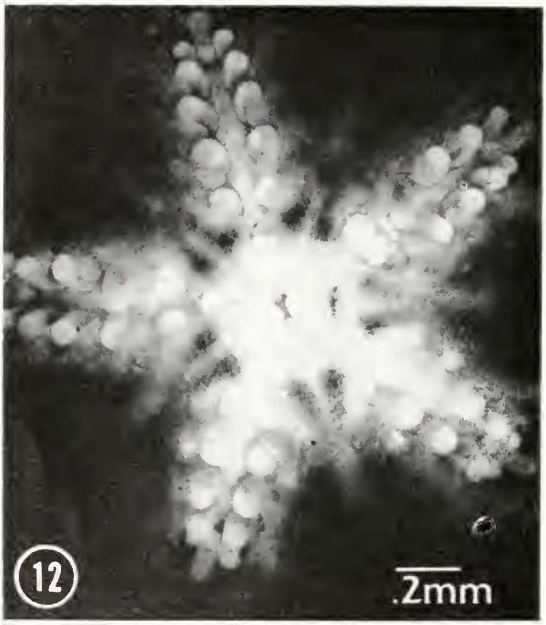


FIGURE 12. Young sea star at 77 days.

Yolk material was completely used up at 35 days and the young sea star appeared translucent. The fifth pair of podia (T) on each ray had developed distal to the fourth pair by this time (Fig. 11). Figure 12 shows the five functional pairs of tube feet and the well developed, muscular mouth of a 77-day-old sea star.

The chronological development as discussed in the preceding text is summarized in Table I.

Echinaster embryos increased in diameter from 0.86 to 0.99 mm within the first $6\frac{1}{2}$ hours after fertilization. By 6 days the larvae reached a length of 1.55 mm (Table II). Growth rates based on ray lengths were employed when larvae attained a sufficient measuring size at 6 days. Juvenile ray lengths showed dramatic increases between 6 and 10 days (0.37 to 0.68 mm) followed by a second burst of growth at 14 days (0.69 to 0.80 mm) (Table III, Fig. 13). Values for the ratio R/r are presented in Table III. Growth rates expressed by wet weights

TABLE I

Chronology of larval development

Time	Developmental events
0 hr	Fertilization
1½ hr	Germinal vesicle breakdown
5 hr	4-cell stage
6½ hr	32-cell stage
8 hr	64-cell stage
10 hr	Morula
23 hr	Blastula; gastrulation
48 hr	Modified brachiolaria larvae with preoral lobe, four small larval arms and sucker
3 days	Five hydrocoel lobes appear; bulges for the first two pairs of tube feet on each ray develop
4 days	Lateral flattening of larval body; hydropore opens to right side of larva
5 days	Differentiation of larval arms and sucker; water ring and radial canals are distinct; bulges of terminal podia appear
6 days	Differentiation of first two pairs of tube feet on each ray
7 days	Tube feet are functional; larval attachment is released; juvenile under own power; terminal podia are differentiated; preoral lobe absorption begins as it folds towards left side of larva
8 days	Formation of third pair of tube feet on each ray; preoral lobe folding completed
10 days	Formation of fourth pair of tube feet on each ray; development of spines on oral and aboral surfaces
14 days	Mouth opens
16 days	Anus opens; preoral lobe appears to be completely absorbed
35 days	Fifth pair of tube feet developed on each ray; yolk materials appear to be used up

were calculated (Table III) and showed a correlation coefficient of 0.85 when compared to growth rates expressed in terms of ray lengths.

DISCUSSION

Fragmentary observations on the external development of *Echinaster echinophorus,* spawning eggs of the buoyant type, were reported by Kempf (1966). Once the embryos of this type completed their free-swimming stage and settled to the substratum, development was comparable to that of the demersal type ob-

TABLE II

Growth rates of early stages of Echinaster echinophorus *expressed as larval lengths (mm) based on a minimum sample size of 10*

Time	Length range	Mean length
1½ hr	0.84–0.88	0.86
5 hr	0.88–1.00	0.91
6½ hr	0.96–1.00	0.99
2 days	0.99–1.26	1.24
3 days	1.20–1.37	1.34
4 days	1.35–1.48	1.48
5 days	1.50–1.54	1.53
6 days	1.48–1.58	1.55

TABLE III

Growth rates of juvenile Echinaster echinophorus *expressed as ray lengths (R, mm) and wet weights (mg) based on a minimum sample size of 10*

Time (days)	R		R / r	Wet weight	
	Range	Mean		Range	Mean
6	0.36–0.40	0.37	1.01	0.1–0.2	0.15
7	0.40–0.56	0.49	1.10	0.1–0.2	0.17
8	0.54–0.60	0.57	1.18	0.1–0.2	0.18
10	0.62–0.72	0.68	1.25	0.2–0.3	0.26
12	0.66–0.70	0.69	1.26	0.3–0.4	0.34
13	0.60–0.72	0.69	1.26	0.3–0.4	0.38
14	0.76–0.84	0.80	1.28	0.3–0.5	0.40
16	0.74–0.84	0.82	1.30	0.3–0.5	0.43
17	0.76–0.84	0.82	1.30	0.3–0.5	0.43
20	0.82–0.92	0.87	1.32	0.3–0.5	0.43
23	0.88–0.90	0.88	1.32	0.5–0.6	0.52
28	0.82–0.98	0.90	1.34	0.5–0.6	0.53
35	0.82–1.00	0.94	1.35	0.5–0.6	0.56
43	0.90–1.00	0.95	1.36	0.5–0.6	0.58
77	0.98–1.10	1.03	1.37	0.8–1.0	0.85

served in the present study. The free-swimming stage which was completed by the end of the third day lasted for 24 hours. The remaining observations of Kempf (1966) are summarized in Table IV.

Kempf's (1966) observations showed the formation of tube feet was much slower (culture temperature at 27° C), with the third pair forming at 10 days compared to 8 days in the present work, and the fourth developing at 23 days, compared to 10 days in the present study. Animals reached a length of 2.2 mm at

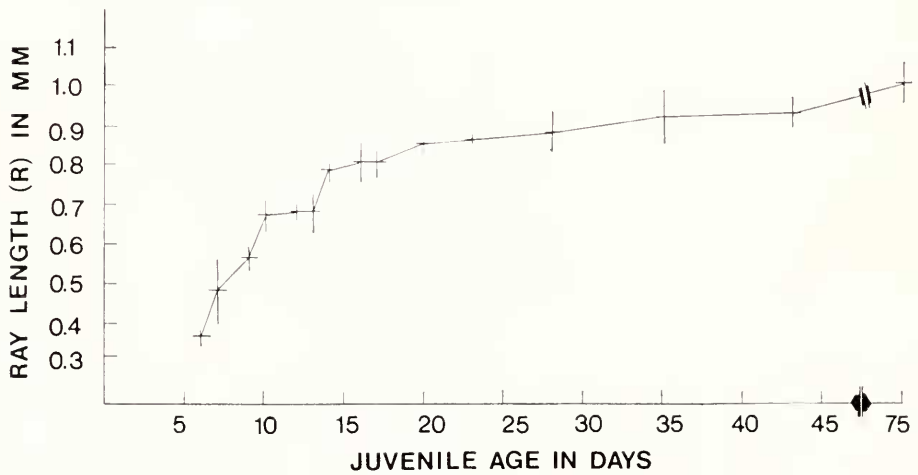


FIGURE 13. Juvenile growth rate of *Echinaster echinophorus*. Vertical lines represent one standard deviation; horizontal lines represent means based on a minimum sample size of 10.

2 days and increased to 3 mm by 4 days. R values enlarged from 0.8 mm to 1.05 mm between 4 and 12 days and reached 1.6 by 100 days. R/r values ranged from 1.26 at 6 days to 1.39 at 100 days (Kempf, 1966). Unfortunately, no comparative data concerning culture conditions, formation of the fifth pair of tube feet, mouth, spines or anus is available.

Löhner (1913) reported that embryos of European *Echinaster sepositus*, measuring 1 mm in diameter when spawned, attained a R of 1.2 mm and a R/r of 1.5 at 20 days of growth. Nachtsheim (1914) observed that eggs of *E. sepositus* were bright red, passed through a free-swimming stage (similar to that described

TABLE IV

Observations on development of Echinaster echinophorus *(Kempf, 1966) and* Echinaster sepositus *(Löhner, 1913; Nachtsheim, 1914)*

Time (days)	Developmental events	
	Echinaster echinophorus (Kempf, 1966)	*Echinaster sepositus* (Löhner, 1913; Nachtsheim, 1914)
3	Growth of larval arms and sucker; larvae settle to substratum	Modified bipinnaria
4	Development of bulges for first two pairs of podia on each ray	
5	Both pairs of podia on each ray are differentiated; water ring and radial canals with primary podia become distinct	Development of four larval arms and sucker; fixation of larvae to substratum
6	Podia become functional; larval attachment is released	Development of bulges for first two pairs of podia and terminal podium on each ray
8–9	Coloration changes from brown to orange	
10	Third pair of podia develop on each ray	Podia are functional; absorption of preoral lobe begins
14		Development of third pair of podia on each ray; absorption of preoral lobe nears completion
23	Fourth pair of podia develop	

by Kempf, 1966) and reached a R of 1.9 mm and a R/r of 1.7 by $7\frac{1}{2}$ months. Development was somewhat slower in the European species which may reflect the influence of lower culture temperatures (Table IV).

Folding of the preoral lobe towards the oral surface of the 7- to 8-day *Echinaster* larva (Figs. 5, 6) has not been observed by previous investigators. Löhner (1913), Nachtsheim (1914) and Kempf (1966) have indicated that absorption of larval tissues took place in an uniform manner disregarding any folding towards either the oral or aboral surface. Soon after larval fixation, the preoral lobe of *Henricia sanguinolenta* (*Cribella oculata*) (order Spinulosida, family Echinasteridae) becomes twisted towards the central disc as the disc rotates in a counterclockwise direction. Through this process the right lateral arm extends backwards towards the right (aboral) larval side as the single dorsal arm twists

with the disc through about 15° (Masterman, 1902). Masterman (1902) viewed this process as a purely mechanical torsion resulting from the folding of the disc towards the oral surface. MacBride (1896) also observed sharp flexure of the disc downwards towards the left larval surface of *Asterina gibbosa* (order Spinulosida, family Asterinidae).

Larval tissues of *Solaster endeca* and *Crossaster papposus* (order Spinulosida, family Solasteridae) undergo a characteristic flexion and torsion at 13 to 19 days of growth (Gemmill, 1912, 1920). A lateral flexion of the preoral lobe towards the oral surface of the embryo and a torsion of the neck of the lobe in a clockwise direction occur simultaneously. Both movements proceed in a gradual manner and are completed by 21 to 22 days after fertilization. The preoral lobe is folded against the left side of the body and is incorporated into the central area of the oral surface through which the mouth will open after another 40 to 50 days. Preoral lobe folding in *Echinaster echinophorus* is not as extensive as that of *Solaster* and *Crossaster* and is restricted to the peripheral tisues between rays number 1 and 5. Neither is there an apparent incorporation of the preoral lobe into the central region of the oral disc of *Echinaster*.

Various invertebrate species have been shown to exhibit different developmental patterns in different regions within their geographic ranges (Thorson, 1950). Thorson (1950) concluded that this phenomenon, poecilogony, is insignificant in the echinoderms. Animals presently classified as *Echinaster echinophorus,* however, shed two characteristically different egg types which undergo different developmental patterns.

The species *echinophorus* in the Florida study area contains at least four morphologically distinct variants. The demersal egg type (Type 2) described in the present study is consistently spawned by the smallest of the variants which measures approximately 6 cm from the tip of a ray to the tip of the opposite ray, occurs in 1–2 meters of water and has a bright red and black coloration pattern. The buoyant egg type (Type 1) has been observed to be spawned by an *echinophorus* which is at least 5 cm larger in diameter, is colored pale orange and normally occurs in slightly deeper water ranging from 3–5 meters in deepth. No spawning has been observed in the remaining two variant types.

Preliminary cross fertilization experiments have shown that mature sperm from either of the two morphological variants will fertilize mature ova of the other. Larval and juvenile development appear to progress normally.

Either this species exhibits differential reproductive behavior in the same locality at the same time, or two or more species are present. This problem can only be solved by completing an extensive taxonomic and developmental study.

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Summary

1. *Echinaster echinophorus* off the west coast of Florida was observed to spawn, from early April to late July, two different egg types: one type is pelagic,

brown to black in color and 1.0–1.3 mm in diameter, while the second is bright orange, ranges from 0.84 to 0.88 mm in diameter and settles immediately to the substratum after spawning.

2. The present study reports on external morphogenesis of *Echinaster* eggs of the second type.

3. Cleavage stages occurred rapidly at the laboratory temperature of 25° C with the gastrula being reached 24 hours after fertilization. By 48 hours a modified brachiolaria develops with 4 larval arms and a central sucker. Folding of the preoral lobe toward the oral surface at 7 days of growth is reported for the first time in *Echinaster*.

4. A chronology of larval and juvenile development is presented.

5. Larval growth rates expressed as lengths (mm) as well as juvenile growth rates expressed as ray lengths (mm) and wet weights (mg) are presented.

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